File No. : 6013-118US

Québec, Canada
April 16, 2008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant :	Université Laval
Serial No. :	10/528,463
Filing date :	March 21, 2005
Title :	METHOD FOR DETERMINING PREDISPOSITION TO A PHYSIOLOGICAL REACTION IN A PATIENT
Group Art Unit :	1634
Examiner :	Amanda Marie Shaw
Agent of record:	Louise G. Bernier, Reg. No. 38,791
Telephone number:	(418) 640-5245

DECLARATION OF CHANTAL GUILLEMETTE

I, Chantal Guillemette, do hereby declare and state as follows:

1. I am a citizen of Canada and I am employed as a full-time Professor, Faculty of Pharmacy, Laval University, in Quebec City, Canada.

2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.

3. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.

4. I am the sole inventor in the present application, I have read and am thoroughly familiar with the contents of U.S. Patent Application Serial No. **Serial No.** :10/528,463 entitled: METHOD FOR DETERMINING PREDISPOSITION TO A PHYSIOLOGICAL REACTION IN A PATIENT, including the claims as originally filed and amended herewith.

5. I have been advised that the Examiner in the office action of January 17, 2008 has rejected all pending claims based on the fact that they fail to comply with the enablement requirement. Specifically, the Examiner contends (articulated) that according to Example 4 and Figure 12 of the present application, the results for this variation between the presence of the -

275 mutated allele (A) and higher glucuronidation rate with SN-38 (*sic*; read MPA) is not statistically significant.

6. Accordingly, I have been asked to provide further data obtained after the filing date of the present application supporting the invention as claimed and advances that the above-identified patent application provides to the medical field.

7. I wish to submit 3 scientific papers (peer- reviewed) which have appeared after the filing date of the present application and that support our data. These papers will be presented and discussed chronologically.

8. Firstly, my group has published additional data in: " *Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver; Pharmacogenetics 2004 Volume 14(8), August 2004, pp 501-515; Girard, Hugo[a]; Court, Michael H[b]; Bernard, Olivier[a]; Fortier, Louis-Charles[a]; Villeneuve, Lyne[a]; Hao, Qin[b]; Greenblatt, David J[b]; von Moltke, Lisa L[b]; Perussed, Louis[c]; **Guillemette, Chantal**[a]".* In this paper, it is stipulated that:

- UGT1A9 expression levels were higher in patients with **variations at positions -275 (P = 0.006)**, -331/-440 (P = 0.046), -665 (P = 0.042) and -2152 (P = 0.0004) [p. 507, 1st col., last para.]
- Significant differences in microsomal UGT1A9 protein content were observed among UGT1A9 genotype groups for comparison between individuals with the wild-type promoter (4023 ± 2083 arbitrary units; the mean expressed is the mean of values *after* reporting the levels of expression relative to the lowest) and subjects with **variation at position -275 (8086 ± 2471 arbitrary units; two-fold higher, P = 0.014)**. This elevation was even more pronounced when the comparison was restricted to individuals with both the -275 and -2152 SNPs (9156 ± 1779; 2.3-fold higher, P = 0.003; Fig. 5a). [p. 507, 2nd col., penultimate para.]
- **Relative levels of UGT1A9 protein were significantly higher in subjects with the -275 (1.4-fold, P = 0.011)** and -275/-2152 (1.6-fold, P = 0.0006) SNPs compared to individuals with other SNPs (non-carriers). **These findings point to the -275 and -2152 variations as the likely cause of elevated UGT1A9 protein levels.** [para. bridging p.507 and 508]
- Compared to individuals with the wild-type homozygous promoter, propofol glucuronidation activities for subjects with the -275 and -2152/-275 SNPs were elevated by 2.2- and 2.3-fold, respectively (Fig. 5b). Similar results were observed for MPA with a 1.9- and 2.1-fold elevated activities for carriers of the -275 and -2152/-275 SNPs, respectively, compared to individuals homozygous for the wild-type promoter (Fig. 5c). [p.508, 1st col., last para.]

9. Secondly, in the following paper from another group: " *Clinical Pharmacology & Therapeutics (2005) 78, 351–361;* Kuypers *et al.* stipulate that:

- In contrast, for patients taking 2 g MMF per day, a significant decrease in MPA exposure was observed in those who **carried either the *T—275A*** or the *C—2152T* promoter region polymorphism (or both) compared with those who did not (MPA AUC_{0-12}, 31.7 17.6 mg h/L versus 63.6 30.9 mg h/L **[*P* = .009]**; C_0, 1.23 1.25 mg/L versus 2.84 1.64 mg/L **[*P* = .04])** (Fig. 1). [p. 355, 1st col., last sentence]

- *UGT1A9* promoter region SNPs were either the most significant independent variable or even the only significant independent variable when MPA AUC_{0-12} (together with gender), MPA AUC_{6-12}, percent enterohepatic circulation and recirculation, C_0 (together with gender and graft function), t_{max}, and CL/F (together with liver dysfunction) were considered. [p.357, 1st col., last sentence of 2nd para.]
- **This study has demonstrated for the first time that *T—275A* and *C—2152T* SNPs of the *UGT1A9* gene promoter region, producing higher in vitro glucuronidation rates,[16] are associated with significantly lower MPA exposure (AUC_{0-12}, C_0) in de novo renal transplant recipients treated with 2 g MMF per day.** [p.358, 1st col.., 1st para. of Discussion]

This same author even goes on to cite my own work:

- **In vitro experiments demonstrated that *T—275A* and *C—2152T* SNPs correlate with higher hepatic expression of UGT1A9 and increased in vitro glucuronidation activity for MPA compared with those of wild-type individuals.[22]** (ref 22 being the preceding paper from my group a.k.a Girard et al.) [2nd para. of Discussion]

10. Thirdly,in the the paper: "Clinical Pharmacology & Therapeutics (2005) 78, 317–321; doi: 10.1016/j.clpt.2005.06.008; Genetic and nongenetic determinants of between-patient variability in the pharmacokinetics of mycophenolic acid; Dennis A. Hesselink MD[1] and Teun van Gelder MD, PhD[1], it is stated that:

- These observations (from Kuypers et al., see supra) demonstrate for the first time that, *in vivo*, interindividual variability in the pharmacokinetics of MPA can be partially explained by genetic variation. **Given the high allelic frequency of** the *UGT1A9* - 2152C>T and **-275T>A SNPs (approximately 15% in white subjects), as well as the 2-fold reduction in MPA exposure in comparison with noncarriers, these findings are also likely to be clinically relevant and offer both a rationale and a means for a personalization of MMF treatment.** [p.319, para. bridging 1st and 2nd col.]

11. In conclusion, in addition to providing additional data that is reproducible and highly statistically significant, the $T^{-275}A$ substitution has been found and validated by other groups independent of my own.

12. Lastly, and as mentioned in the last two papers presented, these findings are likely to be clinically relevant and be applicable to patients who are to be exposed to drugs metabolized by UGT1A9. To support such a contention, I wish to submit the following US patent 6,395,481 (enclosed) which covers the very first method for detecting polymorphism (in UGT1A1) that has been approved by the FDA (see attached Press Release from FDA: http://www.fda.gov/bbs/topics/NEWS/2005/NEW01220.html).

13. I hereby declare that all statements made herein to my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed _____ Dated April 15, 2008
Chantal Guillemette

encl. 3 articles
1 US patent
1 web press release

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Guillemette, Chantal	Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Laval University (Quebec, Canada)	B.Sc.	1992	Biology
CHUL Research Center (Quebec, Canada)	Ph.D.	1996	Endocrinology
Trondheim University, Dr. JR. Idle	PDF	1997	Pharmacogenetics
Massachusetts Institute of Technology, Dr. DE. Housman	PDF	2000	Pharmacogenetics

Professional Experience:

1990-1996 M.Sc. and Ph.D. student in the laboratory of Dr. Alain Belanger, CHUL Research Center
1996-1997 Post-Doctorat, CREMO laboratory, Dr. Fernand Labrie
1997-1997 Post-Doctorat, Trondheim University, Dr. Jeffrey R. Idle
1997-2000 Post-Doctorat, Massachusetts Institute of Technology, Dr. David E. Housman
2000-2002 Assistant Professor, Faculty of Pharmacy, Laval University, Quebec, Canada and Member of the CREMO laboratory
2002- Associate Professor, Faculty of Pharmacy, Laval University, Quebec, Canada and Member of the CREMO laboratory
2003- Chairholder of the Canada Research Chair in Pharmacogenomics

Awards And Other Professional Activities:

1994 Best scientific lecture in Endocrinology and Physiology
1996 Ph.D. with Honors
1997-2000 Postdoctoral fellowship (Medical Research Council of Canada)
1997-1999 Postdoctoral fellowship (Fonds de la Recherche en Sante du Quebec)
2001-2006 Scholarship from Canadian Institute of Health Research (CIHR)
2001-2006 Scholarship from Canadian Institute of Health Research (CIHR and /RxD)
2001-2003 Scholarship from Fonds de la Recherche en Sante du Quebec (FRSQ)
2001-2003 Member of the FRSQ-Health research committee for the evaluation of post-doctoral fellowship application
2001-2002 Member of the committee for the evaluation of doctoral studentship IRSC
2002- Member of the committee for the evaluation of research grant IRSC, Pharmaceutical Sciences
2001- Member of the 'Fondation de l'enseignement et de la recherche' (FER), Faculty of Pharmacy, Laval University
2001- Member of the Ethic committee, Laval University
2001- Member of the Club de Recherche Cliniques du Quebec (CRCQ); ASPET, ISSX
2003- Chairholder of the Canada Research Chair in Pharmacogenomics
2004- President, Scientific committe 'Fondation de l'enseignement et de la recherche' (FER), Faculty of Pharmacy, Laval University

Current Research Awards:

"Canada Research Chair in Pharmacogenomics"
Holder: Chantal Guillemette, PhD
Agency: Canada Research Chair Program and Canadian Institute of Health Research (CIHR)
Type: Individual investigator award (5 years – Level II young investigator)
Period: March, 2003 - March, 2008

"Pharmacogenomics of UGT drug-metabolizing enzymes"
Principal Investigator: Chantal Guillemette, PhD
Agency: Canadian Institute of Health Research (CIHR)
Type: Operating grant (initial 3 years and 5 years renewal)
Period: March, 2000 - March, 2008

"A novel class of UDP-glucuronosyltransferase that control cell metabolism"

Principal Investigator: Chantal Guillemette, PhD
Agency: Natural Sciences and Engineering Research Council of Canada (NSERC)
Type: operating grant (5 years)
Period: April 2007-March 2012

"Metabolic profiling of estrogens in women"
Principal Investigator: Chantal Guillemette, PhD
Agency: Canadian Institute of Health Research (CIHR)
Type: operating grant (Initial 3 years and 3 years renewal)
Period: Oct, 2001- Sept, 2007

"Pharmacogenetics of Mycophenolic acid: role of UGT drug-metabolizing enzymes"
Principal Investigator: Chantal Guillemette, PhD and Mary HH Ensom, Pharm.D.
Agency: Canadian Institute of Health Research (CIHR)
Type: operating grant (3 years)
Period: September, 2004 –September, 2008

"Study of androgen and estrogen formation and metabolism in the human"
Principal Investigator: Fernand Labrie, PhD
Agency: Canadian Institute of Health Research (CIHR)
Type: Group (5 year group award)
Period: October, 2001 - March, 2006

"Génomique fonctionnelle et maladies endocriniennes"
Principal Investigator: Fernand Labrie, PhD
Agency: Canadian Institute of Health Research (CIHR)
Type: MOP (6 years group award)
Period: March, 2002 –February, 2008

Supervisory experience: currently supervise; 1 undergraduate; 2 Master; 5 Doctoral and 1 Post-doctoral

Publications (2003-2007): (underline are students of CG)

Publications	Already published	*Accepted or in the press*
Refereed articles	42	3
Book chapter	1	
Patent	1	
Abstracts	103	
Invited conference	19	

- Bertrand Caillier*, Johanie Lepine*, Jelena Tojcic, Vincent Ménard, Louis Perusse, Alain Bélanger, Olivier Barbier and **Chantal Guillemette** A pharmacogenomics study of the human estrogen glucuronosyltransferase UGT1A3. *co-first authors Pharmacogenetics and Genomics **July 2007** * co-first authors
- Eric Lévesque , Robert Delage, Marie-Odile Benoit-Biancamano, Patrick Caron, Olivier Bernard, Félix Couture and **Chantal Guillemette.** Polymorphisms in UDP-glucuronosyltransferases UGT1A8 and UGT1A9 Result in a Significantly Altered Pharmacokinetic Mycophenolate Mofetil Profiles. Clinical Pharmacology and Therapeutics March **2007**
- Eric Levesque*, Hugo Girard*, Kim Journault, Johanie Lepine and **Chantal Guillemette.** 'Regulation of the UGT1A1 bilirubin-conjugating pathway: role of a new splicing event at the *UGT1A* locus' Hepatology **2007** Jan;45(1):128-38. *co-first authors
- Olivier Bernard, Jelena Tojcic, Kim Journault, Louis Perusse and **Chantal Guillemette**
- Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. Drug Metabolism and Disposition, **2006** June 23
- Jean Thibaudeau , Johanie Lépine*, Jelena Tojcic, Yannick Duguay, Georges Pelletier, Marie Plante, Jacques Brisson, Bernard Têtu, Simon Jacob, Louis Perusse, **Alain Bélanger** and **Chantal Guillemette**. Characterization of common UGT1A8, UGT1A9 and UGT2B7 variants with different capacity to inactivate mutagenic 4-hydroxylated metabolites of estradiol and estrone Cancer Res. **2006** Jan 1;66(1):125-33.
- Jean-François Gagnon, Olivier Bernard, Lyne Villeneuve, Bernard Têtu and **Chantal Guillemette.** Irinotecan inactivation is modulated by epigenetic silencing of UGT1A1 in colon cancer. Clinical Cancer Research, *2006* Mar 15;12(6):1850-8.

- Hugo Girard, Lyne Villeneuve, Michael H. Court, Louis-Charles Fortier, Patrick Caron, Qin Hao, Lisa L. von Moltke, David J. Greenblatt, **Chantal Guillemette.** The novel UGT1A9 intronic I399 polymorphism appears as a predictor of SN-38 glucuronidation levels in the liver. Drug Metabolism and Disposition, **2006** Apr 4; [Epub ahead of print]
- Hugo Girard, Jean Thibaudeau, Michael H. Court, Louis-Charles Fortier, Lyne Villeneuve, Patrick Caron, Lisa L. vonMoltke, David J.Greenblatt, **Chantal Guillemette.** UGT1A1 polymorphisms are important determinants of dietary carcinogen detoxification in the liver. Hepatology, **2005** Aug;42(2):448-57
- Butler LM, Duguay Y, Gagné JF, Millikan RC, Sinha R, Sandler RS, **Guillemette C.** Joint effects between UDP-glucuronosyltransferase 1A7 genotype and dietary carcinogen exposure on risk of colon cancer. Cancer Epidemiol Biomarkers Prev, **2005** Jul;14(7):1626-32.
- Mackenzie PI, Walter Bock K, Burchell B, **Guillemette C,** Ikushiro SI, Iyanagi T, Miners JO, Owens IS, Nebert DW.Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. Pharmacogenet Genomics. **2005** Oct;15(10):677-685
- Yannick Duguay, Monica McGrath, Johanie Lépine, Jean-François Gagné, Susan E. Hankinson, Graham A. Colditz, David J. Hunter, Marie Plante, Bernard Têtu, Alain Bélanger, **Chantal Guillemette***, Immaculata De Vivo. The functional UGT1A1 promoter polymorphism decreases endometrial cancer risk. Cancer Research. **2004** Feb 1;64(3):1202-1207
- Wells, P., P. Mackenzie, R. Chowdhury, **C. Guillemette** and J. K. Ritter. "Glucuronidation and the UDP-glucuronosyltransferases (UGTs) in drug therapy and disease." Drug Metab. Dispos. **2004** Mar;32(3):281-90.
- Yannick Duguay, Cecilie Båår, Frank Skorpen, **Chantal Guillemette*** A novel functional polymorphism in the UDP-glucuronosyltransferase 2B7 promoter that affects morphine glucuronidation in cancer patients. Clinical Pharmacology and Therapeutics. **2004** Mar;75(3):223-33.
- Hugo Girard, Michael H. Court, Olivier Bernard, Louis-Charles Fortier, Lyne Villeneuve, Qin Hao, David J.Greenblatt, Lisa L. vonMoltke, Louis Perusse, **Chantal Guillemette.** Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics. **2004** Aug;14(8):501-515.
- Olivier Bernard and **Chantal Guillemette.** The main role of UGT1A9 in the metabolism of mycophenolic acid and the effects of naturally occurring variants. Drug Metabolism and Disposition, **2004** Aug;32(8):775-778.
- Johanie Lépine, Olivier Bernard, Marie Plante, Bernard Têtu, Fernand Labrie, Alain Bélanger, **Chantal Guillemette.** Specificity and regioselectivity of the conjugation of estradiol, estrone and their catecholestrogen and methoxyestrogen metabolites by human UGTs expressed in endometrium. J Clin Endocrinol Metab. **2004** Oct;89(10):5222-5232.
- Olivier Barbier, Lyne Villeneuve, Virginie Bocher, Coralie Fontaine, Ines Pineda Torra, Christian Duhem, Vladimir Kosykh, Jean-Charles Fruchart, **Chantal Guillemette** and Bart Staels. Hepatic expression of the UGT1A9 gene is governed by HNF4{alpha} Mol Pharmacol, **2004** Oct 6 [Epub ahead of print]
- **Chantal Guillemette**, Alain Bélanger and Johanie Lépine. Metabolic Inactivation of Estrogens in Breast Tissue by UDP-Glucuronosyltransferases. Breast Cancer Res **2004**, 6:246-254 [Epub ahead of print 27 September 2004]
- **Chantal Guillemette*,** Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. The Pharmacogenomics Journal **2003**;3(3):136-58.
- Christopher A. Haiman, Susan E. Hankinson, Immaculata De Vivo, **Chantal Guillemette**, Naoko Ishibe, David J. Hunter, Celia Byrne. Polymorphisms in Candidate Breast Cancer Susceptibility Genes and Mammographic Density. Breast Cancer Research and Treatment **2003** Jan;77(1):27-36.
- Lyne Villeneuve, Hugo Girard, Louis-Charles Fortier and **Chantal Guillemette***. Novel functional genetic variants of the UGT1A7 and UGT1A9 enzymes as potential molecular determinants of irinotecan-induced toxicity. J Pharmacol Exp Ther. 2003 Oct;307(1):117-28. Epub **2003** Aug 27.
- Barbier O, Villeneuve L, Bocher V, Fontaine C, Pineda Torra I, Duhem C, Kosykh V, Fruchart JC, **Guillemette** C, Staels B. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor alpha and gamma target gene. J Biol Chem. **2003** Apr 18;278(16):13975-83